

Remarks

By the present invention there are provided methods for detecting the presence of oligonucleotides and monitoring oligonucleotide production during amplification. The methods use fluorescence energy transfer by competitive hybridization, wherein competitive hybridization occurs between a fluorophore labeled first probe and target nucleic acid, and between the first probe and a quencher labeled second probe. Fluorescence signal due to the first probe fluorophore increases as target nucleic acid competes away the first probe from binding to, and being quenched by, the second probe.

Claims 2-11 and 14-22 are pending and under active prosecution. The **Listing of Claims** with appropriate status identifier begins on page 2 of this communication. Applicant respectfully requests reconsideration of the present application in view of the reasons that follow.

By the present communication, Claim 20 is amended to define Applicants' invention with greater particularity. No new matter is introduced by the amendment to Claim 20. Specifically, Claim 20 is amended to provide that the claimed first and second probes are of unequal length, support for which is found throughout the specification, e.g., page 1, lines 3-5; page 1, lines 25-27; page 6, lines 16-18. Claim 20 is further amended for conform the claim language; i.e., insertion of the the word "and" between the two steps of the claimed method.

Rejections under 35 USC § 103

To establish a *prima facie* case of obviousness, three criteria must be met: there must be some motivation or suggestion, either in the cited publications or in knowledge available to one skilled in the art, to modify or combine the cited publications; there must be a reasonable expectation of success in combining the publications to achieve the claimed invention; and the publications must teach or suggest all of the claim limitations. See MPEP §2142. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 493; 20

USPQ2d 1438, 1442 (Fed. Cir. 1991); see also MPEP §2142. In analyzing obviousness, the Court of Appeals for the Federal Circuit has repeatedly cautioned that:

[t]he factual inquiry... must be based upon objective evidence of record.... [T]he best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.... [P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

In re Sang-Su Lee, 277 F.3d 1338, 1343 (Fed. Cir. 2002), 61 USPQ2d 1430, 1433 (internal citations omitted).

The 103(a) Rejection Over Tyagi in view of Diamond

The rejection of claims 2-11 and 19-22 under 35 USC § 103(a) as allegedly being unpatentable over Tyagi *et al.* (US 6,103,476) in view of Diamond *et al.* (US 4,766,062) is respectfully traversed.

As acknowledged by the Examiner (Office Action, page 4, lines 1-2), Tyagi *et al.* does not disclose a probe having the features recited in claims 20, 22, and 3-10. In an attempt to meet this missing claim element, the Examiner asserts (Office Action, page 4, lines 3-5) that “Diamond *et al.* discloses a diagnostic reagent containing a complex of a probe (See the Abstract). The probe has the same features as recited in claims 20, 22, and 3-10 (See column 6, lines 3-19, column 21, lines 15-52).”

The Examiner cites to a motivation to combine the references based on alleged solubility of the probe, monitoring of amplification in solution, and alleged mechanism of probe action (Office Action, page 4, lines 6-13, citing to Diamond *et al.* Col. 7, lines 57-67; Col. 8, lines 55-60). As addressed below, Applicants will demonstrate that motivation to combine is lacking and, when fully considered, the art actually teaches away from the combination of the art cited.

Tyagi *et al.* describes a class of probes referred to as “unitary” probes, which comprise two types, a bimolecular probe and a unimolecular probe. The bimolecular probe described by

Tyagi *et al.* is made up of two separate polynucleotides (see Figs. 1 and 2 of Tyagi *et al.*) while the unimolecular probe of Tyagi *et al.* is a single polynucleotide (see Figs. 3-5). (“[p]robes according to this invention having interactive labels are ‘unitary’ by which we mean either bimolecular probes linked as a pair and operable in an assay as linked, or unimolecular, single strands” (Tyagi *et al.*, Col. 4, lines 59-62; emphasis added). Thus, the bimolecular probe of Tyagi *et al.*, which comprises two strands with “interactive labels” broadly encompasses the probe pair combination of Diamond *et al.*, (see Col. 6, lines 3-19), upon which the present rejection is based.

Contrary to the asserted basis for motivation to combine, Tyagi *et al.* repeatedly discloses that bimolecular probes (which would include those by Diamond *et al.*) are not suitable for use in methods of amplification (e.g., PCR) in contrast to unimolecular probes. Specifically, the Examiner’s attention is drawn to Tyagi *et al.* (Col. 6 lines 58 to Col. 7, line 3) which discloses that

[a]ssays according to this invention using probes with interactive labels may include contacting at least one unimolecular probe of the invention with amplification or other nucleic acid synthesis reactions...Bimolecular probes, as stated above, are not suitable for use in any reaction, e.g., PCR, in which the affinity pair would be separated in a target-independent manner” (emphasis added).

Tyagi *et al.* further discloses (Col. 20, lines 47-49) that “[f]or assays wherein the unitary probes will be subjected to melting or other denaturation, the probes must be unimolecular (emphasis added).” As discussed above, Tyagi *et al.* discloses unimolecular and bimolecular probes, the latter encompassing the probes of Diamond *et al.* Thus, Tyagi *et al.* again teaches away from the use of bimolecular probes for assays comprising melting (i.e., amplification).

Tyagi *et al.* yet further discloses (Col. 6, lines 32-34) that “for assays that include a step or steps that may separate the affinity pair in a target-independent manner, only unimolecular probes are suitable” (emphasis added). This means to one of ordinary skill in the art that in steps wherein the probes are separated (i.e., melting), only unimolecular probes are suitable.

Additionally, Tyagi *et al.* provides a rationale based on physiochemical considerations for why bimolecular probes should be avoided for amplification reactions (Col. 3, lines 11-18):

[t]hese strand-displacement probe complexes have drawbacks. The mechanism is two-step, in that the probe complex must first bind to the target and then strand-displacement, via branch migration, must occur before a target is recognized and a signal is generated. Bimolecular probe complexes are not reported to form with high efficiency, resulting in probe preparations wherein the majority of the target binding regions may not be annealed to a labeled strand.

As would be readily understood by the skilled artisan, this physiochemical rationale is based on the kinetics and thermodynamics (i.e., “form with high efficiency”) of biomolecular probe complex formation. Specifically, based on theoretical reaction rate considerations, an intramolecular reaction involving a fluorophore and quencher on the same molecule (i.e., unimolecular) occurs at a significantly faster rate than the corresponding intermolecular reaction (i.e., bimolecular). Thus, Tyagi *et al.* specifically teaches away, by both express statement and theoretical considerations, from using bimolecular probes such as those disclosed by Diamond *et al.* and relied upon by the Examiner in the present rejection. As such, the references are not suitable for combination as asserted.

Furthermore, the assertion (Office Action, page 4, lines 6-13) that one of ordinary skill in the art would have been motivated to apply the complex of the probe of Diamond *et al.* to the invention contemplated by Tyagi *et al.* ignores the fact that the disclosure by Tyagi *et al.* provided a new methodology (i.e., real time monitoring in PCR), which was unknown to Diamond *et al.* Indeed, the probes of Diamond *et al.* are contemplated for use in static hybridization reactions, whereas Tyagi *et al.* discloses a dynamic system having e.g. the unique temperature, concentration and timing considerations associated with real-time PCR, wherein a reaction mixture is repeatedly cycled with a defined sequence of heating and cooling steps, all having defined durations. This begs the question, why Tyagi *et al.* did not consider bimolecular probes such as those disclosed by Diamond *et al.* for use in real time amplification. As discussed above, consideration of the kinetics of formation of fluorophore-labeled probe and quencher-

labeled probe in complex led Tyagi *et al.* to repeatedly teach away from the use of bimolecular probes such as those disclosed by Diamond *et al.*

Furthermore, the Examiner has pointed to no teaching or suggestion in either reference that use of the probes such as those of Diamond *et al.* would have any expectation of success in the new methodology of Tyagi *et al.* The various statements by Tyagi *et al.*, which clearly teach away from using bimolecular probes for amplification, also strongly rebut any possibility for a reasonable expectation of success. Thus, at best the present rejection is a classic case of impermissible hindsight where the invention has been broken into minor elements (i.e., soluble probe, nucleic acid amplification, and proposed mechanism of probe action) that can be found in the art and then reassembled using the teachings of the instant application as a guide. *In re Sang-Su Lee (supra)*.

Accordingly, as there is no motivation to combine the references while in fact there is a teaching away from the invention, and there is no reasonable expectation for success, reconsideration and withdrawal of the rejection are respectfully requested.

The 103(a) Rejection Over Tyagi in view of Diamond, further in view of Hiroaki

The rejection of claims 14-18 under 35 USC § 103(a) as allegedly being unpatentable over Tyagi *et al.* (US 6,103,476) in view of Diamond *et al.* (US 4,766,062), further in view of Hiroaki *et al.* (EP 0461 863 A1) is respectfully traversed. As acknowledged by the Examiner (Office Action, page 4, lines 18-20), Tyagi *et al.* and Diamond *et al.* do not disclose that the target polynucleotide comprises hepatitis C virus genome, that the probe has the sequence of SEQ ID NOs: 3 and 4, or that the primer has the sequence of SEQ ID NOs: 1 and 2. The Examiner asserts that Hiroaki *et al.* discloses the nucleotide of the 5' noncoding region comprising SEQ ID NOs: 1 and 3 and the complementary sequence of SEQ ID NO:2 and base pair 1-17 of SEQ ID NO:4 (Office Action, page 5, lines 3-5).

Applicants respectfully submit that no *prima facie* rejection has been stated because Hiroaki *et al.* fails to cure the defects in the rejection of Tyagi *et al.* and Diamond *et al.* as discussed above. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. In the event that any matters remain to be solved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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